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DEPARTMENT:MLS 400l

Assignment Title: Biomedical Engineering

Course code: MLS410

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Question:

1 Discuss the physics of light and microscope diagram and illustrations needed.

2. Write notes on the biomedical equipment.Add notes on the principle,brand,care and maintenance and cost of,

- Centrifuge
- Automatic tissue processor
- Microtome

Answers

Microscope

Microscope is an optical instrument that uses lens or combination of lens to produce magnified images that are too small to seen by unaided eye. Microscope

provides the enlarged view that helps in examining and analyzing the image. Microscope can be separated into optical theory microscopes (Light microscope), electron microscopes (eg.TEM, SEM) and scanning probe microscopes. (eg.AFM, PSTM).

Optical microscopes function on the basis of optical theory of lenses by which it can magnifies the image obtained by the movement of a wave through the sample. The waves used in optical microscopes are electromagnetic and that in electron microscopes are electron beams. Light microscopes can be classified into Bright field microscope, Phase contrast microscope, Dark field microscope and Fluorescence microscope.

- Light Microscopy

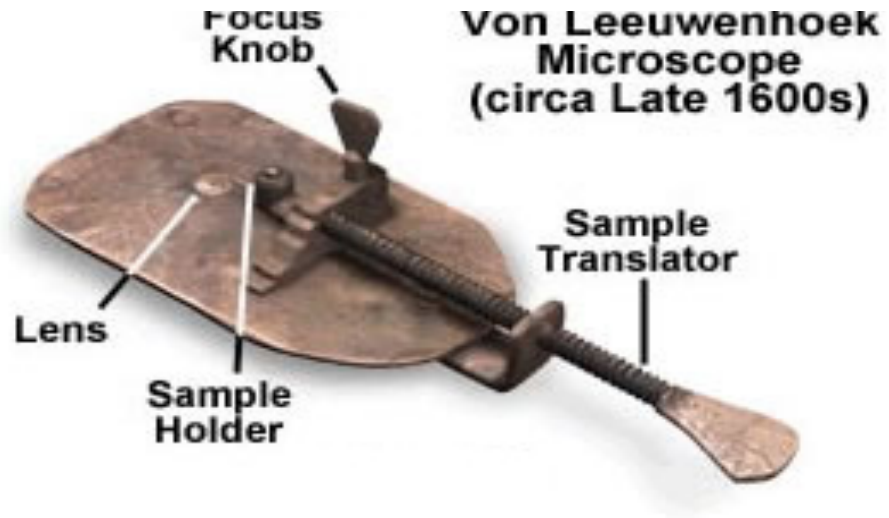
Light microscope uses the properties of light to produce an enlarged image. It is the simplest type of microscope. Based on the simplicity of the microscope it may be categorized into:

A) Simple microscope.

B) Compound microscope.

A. Simple microscope :It is uses only a single lens, e.g.: hand lens. Most of these are double convex or

planoconvex lens. The developments of advanced techniques for grinding and shaping lenses allowed professionals such as Hans Janssen and Anton van Leeuwenhoek to develop simple microscopes which advanced the study of biology significantly.



B. Compound microscope :In the compound microscope, used two lenses or lens systems. One of the lens system formed an enlarged image of the object and the second lens system magnifies the image formed by the first. The modern compound microscope consists of two lens system, the objective and the ocular or eye piece. The first magnified image obtained with objective lens, is again magnified by the eye piece to give a virtual

inverted image. The total magnification the product of the magnifications of two lens systems.

Parts of a Microscope It consists of mainly three parts;

1. Mechanical part - base, c-shaped arm and stage.
2. Magnifying part - objective lens and ocular lens.
3. Illuminating part - sub stage condenser, iris diaphragm, light source.

① Mechanical part

1. Base: It helps in holding the various parts of microscope. It also contains the light source.
2. C-shaped arm: It is used for holding the microscope. And which is connected the eyepiece to the objective lens.
3. Mechanical stage: It is a rigid platform on which specimen to be viewed is placed. It has an aperture at the centre to permit light to reach the object from the bottom. The object on the slide can be moved either sideways or forward and backward with the help of the positioning knobs.

② Magnifying part

Eye piece (ocular lens) : It is time the lens where the final image of the object is viewed. Usually; these lenses have a magnification of either 10X or 15X.

Objective lens:

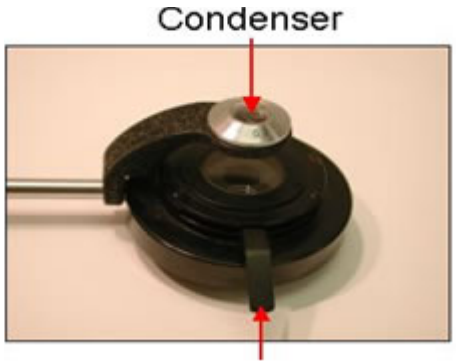
There are three types of objective lens: 4X (scanning objective)

1. 10X (Low power objective lens).
2. 40X (High power objective lens).
3. 100X (Oil immersion objective lens).

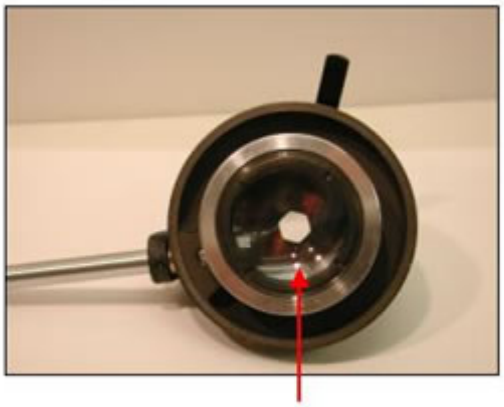
Each objective lens is represented by a particular color. Here we represents 4X with red band, 10X with yellow, 40X with blue and 100X with white. These objective lenses are fitted on to the revolving nose piece. The working distance of an objective is defined as the distance between the front surface of the lens and the cover glass surface or the specimen when it is in sharp focus.

③ Illuminating part

1. Sub stage condenser: It is seen below the stage and made up of a system of convex lenses which focus light from illuminating sources and is used to condense light towards the object. Lowering the condenser diminishes illumination whereas raising the condenser increases the illumination.



2. Iris diaphragm: It is seen immediately below the condenser and operated by small lenses which protrude to one side. Opening and closing of iris diaphragm controls the light reaching the object.



Iris Diaphragm

3. Light source: Light source is situated at the base of the microscope. It is controlled by an ON /OFF switch and a lamp rheostat. Tungsten-halogen lamps are highly reliable light source used in the light microscope. It generates a continuous distribution of light across the visible spectrum.

4. Adjustments Knobs in the Microscope

a) Coarse Adjustment Knob: objective lenses can be moved towards or away from the specimen by using this coarse adjustment knob

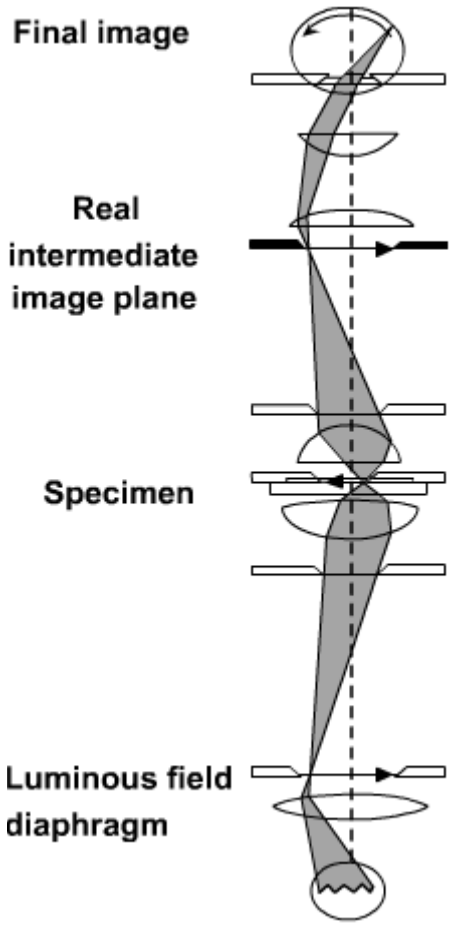
b) Fine Adjustment Knob: It is used to fine tune the focus on the specimen and also used to focus on various parts of the specimen. commonly one uses the coarse

focus first to get close and moves to the fine focus knob for fine tuning.

c. Image formation

The direct or undeviated light from a specimen is projected by the objective and it spreads evenly across the entire image plane at the diaphragm of the eyepiece. The light diffracted by the specimen is come to focus at different localized sites on the same image plane, and the diffracted light causes destructive interference. One of the consequences is the reduction in light intensity resulting the greater or lesser dark areas. The patterns of light and dark that are recognized as an image of the specimen. Because our eyes are very sensitive to variations in brightness, and then the image becomes more or less faithful reconstitution of the original specimen.

The objective lens at first formed a real and inverted magnified image. And then the eye piece further magnifies the same image to virtual magnified image.



Focusing On Microscopic Objects

- **Start with Clean Lenses:**

It is important that microscope lenses be very clean. Before viewing through a microscope, use lens paper to gently clean the lenses.

- **Begin at Low Power Magnification:**

Always begin by viewing the object through a low power lens. Depending on how small the object is, start with the scanning or low-power objective.

Using low-power objective lens, get the target object centered in the field-of-view and focus as much as possible, first by using the coarse focus and then fine-tuning the clarity of the image with the fine focus.

Once the object is in focus, switch to the next higher objective power. Do not change the focus or manipulate the focus knobs in any way while changing objectives.

- Adjustments for oil immersion objective:

Without changing the adjustment of high power, turn to oil immersion objective. One drop of oil is added into on the slide. The nose piece is turned such that the oil immersion objective touches on the drop of oil. Open the iris diaphragm completely. Use only fine adjustments for focusing.

- The Importance of Par focal:

A set of objectives on a microscope are said to be par focal if the viewer can change from one to another and still have the specimen nearly in focus. This is a very convenient feature, because as the magnification

increases, even small manipulations of the focus knob can take a specimen far out of focus.

After changing to a higher objective (such as high-dry or oil-immersion) the viewer needs only manipulate the fine focus knob. Never manipulate the coarse focus at oil immersion. Manipulating the coarse focus at high power can smash the lens into the slide, potentially damaging the scope and the specimen.

- Key Points

Magnification:

Magnification is defined as the degree of enlargement of an object provided by the microscope. Magnification of a microscope is the product of individual magnifying ability of ocular lens and objective lens.

Magnification of ocular lens	Magnification of objective lens	Total magnification
10X	4X	40X
10X	10X	100X
10X	40X	400X
10X	100X	1000X

➢ Resolving power:

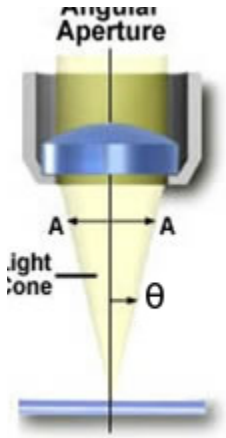
It is defined as an ability to distinguish between two particles situated very close.

➢ Numerical aperture: It is defined as the property of lens that decides the quantity of light that can enter. The angle of the cone of light entering an objective is known as theta.

NA=nsinθ ; NA=numerical aperture

n=refractive index of the imaging medium between the front lens of the objective and the specimen cover glass, a value that ranges from 1.00 for air to 1.51 for specialized immersion oils.

θ= one-half of the angular aperture (A)



➢ Limit of resolution:

It is defined as the shortest distance between two objects when they can be distinguished as two separate entities.

➢ Principle Of a microscope

Microscope is a delicate instrument which should be properly used. Fungal growth on the lens or scratches caused by dust can ruin the lenses. So microscope should be handled carefully.

1. Carry the microscope by holding the C-shaped arm with one hand and other hand under the base. Never swing the microscope while carrying.
2. Never allow direct light to fall on the microscope. Cover the microscope with a plastic cover when not in use.
3. While using oil immersion objective, do not adjust the coarse screw.
4. Oil immersion objective should be cleaned after use by wiping with soft cotton cloth or lens paper.
5. Dry objective should never come in contact with oil.
6. At the end of every experiment, clean the lenses with lens paper.

7. The scanning objective or the 4x objective should be locked in place in the revolving nose piece, the stage should be centered and objectives should be rolled up away from the stage, when the microscope is replaced after use.

8. When the microscope is replaced in the cabin the microscope's arm/pillar must face the opening of the cabin.

Question 2

2. The laboratory centrifuge is a piece of equipment, used mainly in the clinical laboratories to separate the components of body fluids such as blood and urine through centrifugal force. Laboratory centrifuges work by the sedimentation principle, where the centripetal acceleration is used to separate substances of greater and lesser density. In hospital clinical laboratories, benchtop laboratory centrifuges are mostly used. These are commonly referred to as general purpose centrifuges as they offer versatility and convenience and can be equipped in many ways to meet a broad range of clinical requirements, making them a cost-effective solution for many laboratories. There are various types of centrifuges

that depend on the size and the sample capacity. The laboratory centrifuge finds applications in clinical laboratory, histology, hematology ,immunology, microbiology, pathology, serology, and toxicology in most laboratories from academic to clinical to research fields.

Principle of centrifuge

The acceleration at centripetal force causes denser substance to separate out along radial direction at the bottom of the tube.In a solution particles whose density is higher than that of the solvent sink(sediment), and particles that are lighter than it float on top, the greater the difference in density, the faster they move

Types of centrifuge depends on:

- Speed of sedimentation(ultra centrifuge or high speed centrifuge)
- Presence/Absence of vacuum(ultra centrifuge /small bench top)
- Temperature control refrigeration
- Volume of sample capacity
- Depending on the particular application,centrifuges differ in overall design and size.
- A common feature in all centrifuge in central motor

spin a rotor containing the samples to be separated.

Small bench top

- With or without refrigeration
- Slow speed (e.g up to 4000 RPM)
- Common in clinical laboratory (blood,plasma,serum separation)
- Can take aprox (up to)100 tubes depending on diameter

Micro centrifuge (”microfuge “,Eppendrof”)

- Sample volume is small in eppendrof tube
- Refrigerated with or without
- Centrifuge maximum aprox 10000g
- Take tube small volume up to 2ml
- Commonly used of concentration protein.

High speed centrifuge

- Refrigerated
- Use of protein precipitates,large intact organelles cellular debris from tissue homogenization and micro organisms
- They operate maximal centrifugal force of approx 50000g

- Use for research application
- Differential separation of nucleus,mitochondrial, protein, precipitate,e.t.c

Ultra centrifuge

- Refrigerated and evacuated
- The detail biochemistry analysis of sub cellular structures and isolate bio molecules
- Operate at up to 90000g.

Maintenance of a centrifuge

Daily maintenance

- Wipe the inside of the bowl with disinfectant solution and rinse thoroughly
- The centrifuge must not be used if the interior is hot,if unusual vibration or noise occur or if the deterioration (corrosion of parts) is detected.
- A qualified service technician should be contacted.
- Most vibrations are due to improper balancing and can be corrected by rebalancing the buckets and tubes.

Monthly maintenance

- Clean the centrifuge housing,rotor chamber,rotors, and rotor accessories with a neutral cleaning agent
- Clean plastic and non-metal parts with a fresh solution

of 0.5% sodium hypochlorite.

Care of a centrifuge

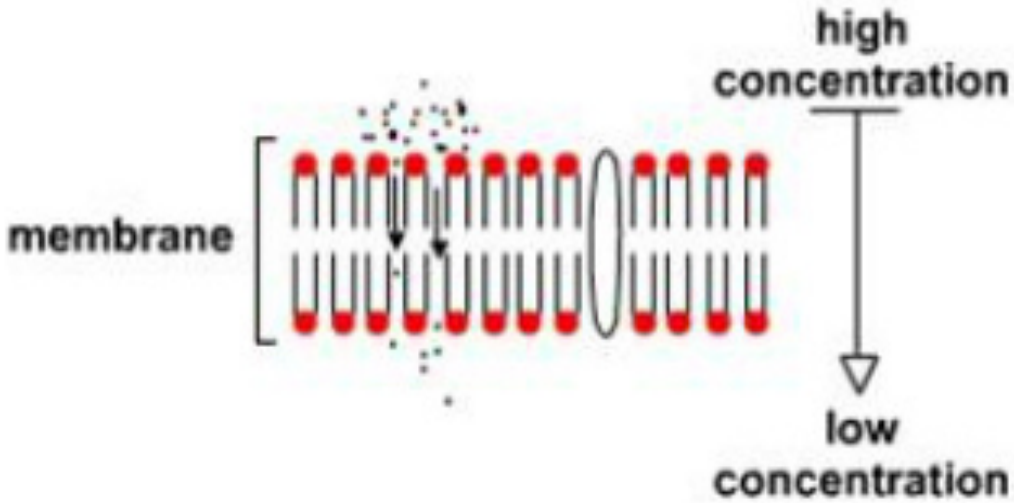
- Reading the manufacturer instructions.
- Placing a centrifuge on a firm level bench out of direct sunlight,towards the back of the bench
- Whenever possible using plastic tubes made of polystyrene or autoclavable.
- Always balancing tubes that are being centrifuged
- Never open the centrifuge when is still spinning.Never try to slow it do with your hand.
- Most centrifuge have a brake, use it only when appropriate.

2. Automatic tissues processor

A tissue processor is a device that prepares tissue samples for sectioning and microscopic examination in the diagnostic laboratory.Microscopic analysis of cells and tissues requires the preparation of very thin, high quality sections (slices) mounted on glass slides and appropriately stained to demonstrate normal and abnormal structures.

The ATP machine plays a big role in the preparation of the tissue by passing them through various chemicals; a

major process called TISSUE PROCESSING . Tissue processing is concerned with the diffusion of various substances into and out of porous tissues. Diffusion results from the tendency of processing reagents to equalize concentrations both inside and outside blocks of tissue. The reagent molecules diffuse down a concentration gradient and move from where they are at a high concentration to where they are at a lower concentration. This movement requires no energy since it always progresses down the concentration gradient.



The rate of diffusion is measured by Fick's law which states that:

$$\text{Rate of diffusion} \propto \frac{\text{Area of diffusion surface x Difference in concentration}}{\text{Thickness of surface over which diffusion takes place}}$$

(where α signifies 'proportional to')

There are significant variables which need to be considered when processing tissue and these include the operating conditions (such as temperature), the concentration of the reagents and the properties of the tissue. In the histology laboratory, conventional tissue processing describes the stages required to take fixed tissue samples through dehydration and clearing to the state where it is completely infiltrated and embedded with a suitable medium (normally paraffin wax) in readiness for cutting sections on a microtome (microtomy). For routine purposes, tissues are most conveniently processed through dehydration, clearing and infiltration stages automatically. There are two broad types of automatic tissue processors available - tissue transfer and fluid transfer types.

Steps in automatic tissue processor

i) FIXATION – this is the process of preserving or fixing tissues by passing them through chemicals called fixatives. The fixatives will help protect the tissue from decay and autolysis. Routine fixative of use is 10% formalin

(ii) DEHYDRATION – this is the process of removing water molecules from the tissue by passing the tissue through ascending grades of alcohol. E.g methanol, acetone, 70-100% alcohol

(iii) CLEARING – this is the process of removing alcohol from the tissue by passing it through chemicals that will remove the alcohol molecules. These agents are called clearing agents. Xylene is mostly used for clearing.

(iv) INFILTRATION – this is the process of filling intracellular spaces left in the tissue by paraffin wax. This will help confer a bit of rigidity to the processed tissue.

(v) EMBEDDING- this last step is manually done. This has to do with immersing the processed tissue into a mould

containing liquid paraffin wax. This is for external support so that the tissue won't crumble during microtomy

Principle of automatic tissue processor

Time required for tissue processing may be considerably reduced when tissue is suspended in fluid,continuously agitated ,Moved from one reagent to another whenever desired,not restricted by working hours.

Maintenance of tissues processor

- Clean outside of instrument with xylene dampened cloth.
- Close retort chamber and press clean for the clean cycle to start automatically
- A quality control chart is recommended to ensure the reagents are monitored and changed frequently as per manufacturers instruction.

Care taken for an automated tissue processor

- Any spillage or overflow should be cleaned immediately
- Accumulation of wax on any surface should be removed

- The temperature of the paraffin wax bath should be set to30•C above the melting point of wax
- Timing should be checked when placing cassettes in the processor.

3. Microtome:

Microtome is an instrument with the help of which sections of tissues are cut and the process of cutting thin sections is known as Microtomy. The thickness of sections produced during microtomy may be between fractions of 50-100 nm, in ultramicrotomy, to several 100 microns. The common range is between 5-10m but both the maximum and minimum thickness is limited by the consistency of relation of the thickness of sections to the nature of tissues. These sections are stained using suitable staining techniques followed by observing them under the microscope.

Principle of a microtome

microtome is a sectioning instrument that allows the cutting of extremely thin slices of a material known as section . microtome are used in microscopy , allowing for the preparation of sample for observation under

transmitted light or electrons radiation . it is a method for the preparation of thin section for materials such as bones, minerals, and teeth.

types of microtome

1.) Rotary microtome

The Rotary microtome is so-called because of a Rotary action of the hand wheel responsible for the cutting moment. The block holder is mounted on a steel carriage, which makes up and down in groves this type of instrument is the most ideal for routine and research work it is excellent for cutting serial sections.

parts of the rotary microtomes

I . Block holder

II . Knife clamp screw
III . Knife clamps
IV . Block adjustment
V . Thickness gauge
VI . The angle of tilt adjustment
VII . Operating handle

2.) Sliding or Base Sledge Microtome

This is a large heavy instrument with a fixed knife beneath which the object moves mounted on a heavy sliding base containing the feed mechanism used primarily for cutting the sections of cellulose nitrate embedded tissues with an obliquely set knife.

Parts of Base-sledge microtome

- Angular tilt adjustment
- Knife clamps
- Block holder
- Coarse feed adjustment
- Operating handle
- Thickness gauge
- Adjustment locking nut
- Block adjustment screw
- Split nut clasp

3.) Cambridge rocking microtome

The instrument is so named because the arm has to move in a rocking motion while cutting the sections. The instrument was invented by Sir Horace Darwin in 1881

was developed by Cambridge company hence it is called the Cambridge rocking microtome. It is a simple machine in which the knife is held by means of microtome thread. The rocking microtome was designed primarily for cutting paraffin wax sections but in an emergency use frozen section by inserting a wooden block in which the tissue is frozen.

Parts of the rocking microtomes

- Knife holder
- Block holder or chuck
- Upper arm
- Screw
- Lever
- Pawl
- Ratchet wheel
- Mil head microtome screw
- Sleeve
- Lower Arm
- Scale

4.) Freezing microtomes

This type has been designed for the production or preparation of frozen sections of fluid and non-fluid tissues usually without preliminary embedding. The object stage is connected to the cylinder of compressed carbon dioxide for the rapid cooling of the tissues and provisions are also made for the cooling of the knife.

Part of freezing type microtome

- Knife clamps
- Operating handle
- Thickness gauge
- Stage
- Stage valve
- Coarse adjustment

Care and maintenance of a microtome

- Dust accumulation must be prevented by putting a cover when not in use
- Wipe the moving parts regularly with good neutral oil(e.g coconut oil) to lubricate and avoid rust.
- After cutting , clean, frequently from accumulated paraffin using a soft brush with xylene.

- Never adjust screws too tightly that may cause binding.